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Short Note

3-{4-[(*E*)-{4-[(*E*)-Phenyldiazenyl]phenyl}diazenyl] phenoxy}propane-1,2-diol

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Abstract: Title compound was designed to be a black quencher of pyrene fluorescence. It was made amphiphilic to serve as a membrane-bound probe. The synthesis is a two-step procedure. The first step is a Mitsunobu reaction of [{(phenyldiazenyl)phenyl}diazenyl]phenol with 1,2-*O*-isopropylideneglycerol. The second step is the cleavage of the isopropylidene protecting group. The title compound has the extinction coefficient 59,000 at λmax = 380 nm. The Forster distance between the title compound and the pyrene was found to be 37.8 Å.

Keywords: membrane probes; black quencher of fluorescence; pyrene

1. Introduction

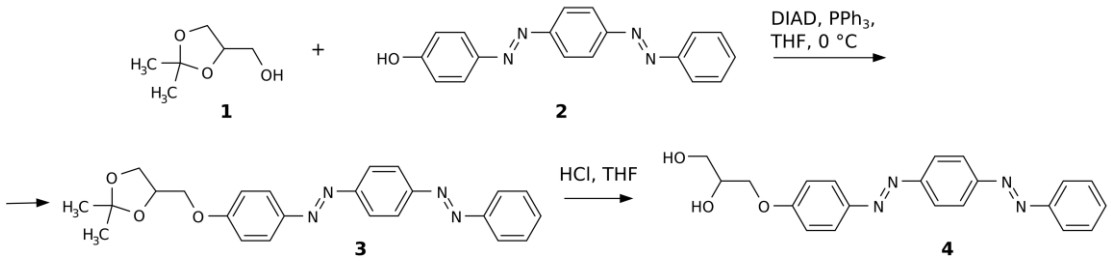
The use of FRET (Förster Resonance Energy Transfer) is a common way to detect interactions between biomolecules. It is based on the ability of fluorescent probes (donors) to transfer excitation energy to appropriate quenchers (acceptors). The energy transfer occurs only if a donor and an acceptor are close enough to each other (the energy transfer efficiency at the Förster distance is 50%). Thus, if two molecules are labeled with a donor and an acceptor, respectively, their interaction can be detected by monitoring the fluorescence intensity of the donor. A pair of a labeled protein (e.g., by a donor) and a labeled lipid (e.g., by an acceptor) or just two labeled proteins is commonly used when studying protein/membrane interactions (recent examples are described in [1–5]).

Alternatively, both the donor and the acceptor can be placed inside the lipid membrane. In this case, FRET will occur between the probes distributed in the membrane: any change in the distribution of probes affects the energy transfer efficiency, and thereby changes the fluorescence intensity of the donor. FRET between membrane-bound probes was extensively used to study the lateral organization of membranes [6–8] and lipid transporting enzymes (for example, probes used in [9] and [10]).

In the course of our development of membrane-bound probes and specific quenchers of their fluorescence [11,12], we have faced the necessity of designing a new membrane-bound black quencher specific to pyrene fluorescence—a quencher for pyrene and pyrene-based lipid probes. Here we report the synthesis of this black quencher.

2. Results and Discussion

The title compound (**4**, Scheme 1) is designed to be an amphiphilic molecule. The nonpolar diazen chromophore represents a hydrophobic core. The glycerol moiety represents a hydrophilic head. The length of the hydrophobic core is 16 carbon atoms, which is close to the length of fatty acid residues in phospholipids (usually 16–18).



Scheme 1. Synthesis of 3-(4-[(*E*)-{4-[(*E*)-phenyldiazenyl]phenyl}diazenyl]phenoxy) propane-1,2-diol.

Due to the amphiphilic structure, the compound is supposed to penetrate into the lipid membrane and remain in it, with its hydrophilic head located at the membrane polar region. At the same time, the title compound is not a phospholipid. This is important, since the quencher should not compete with phospholipids in binding to specific enzymes. The dianix yellow 5R was chosen as a chromophore because of its spectral properties (see below).

The dianix yellow 5R (**2**) is converted to ether (**3**) through Mitsunobu reaction [13] with 1,2-*O*-isopropylideneglycerol (solketal, **1**) (Scheme 1). Removing of the acetonide protecting group with an acid yields the title compound (**4**).

Title compound has absorbance maximum λmax = 378 nm and its extinction coefficient is 59,000 (at 378 nm). The absorbance spectrum of the title compound perfectly overlaps with the emission spectra of pyrene (Figure 1). The Förster distance for the title compound and pyrene is 37.8 Å.

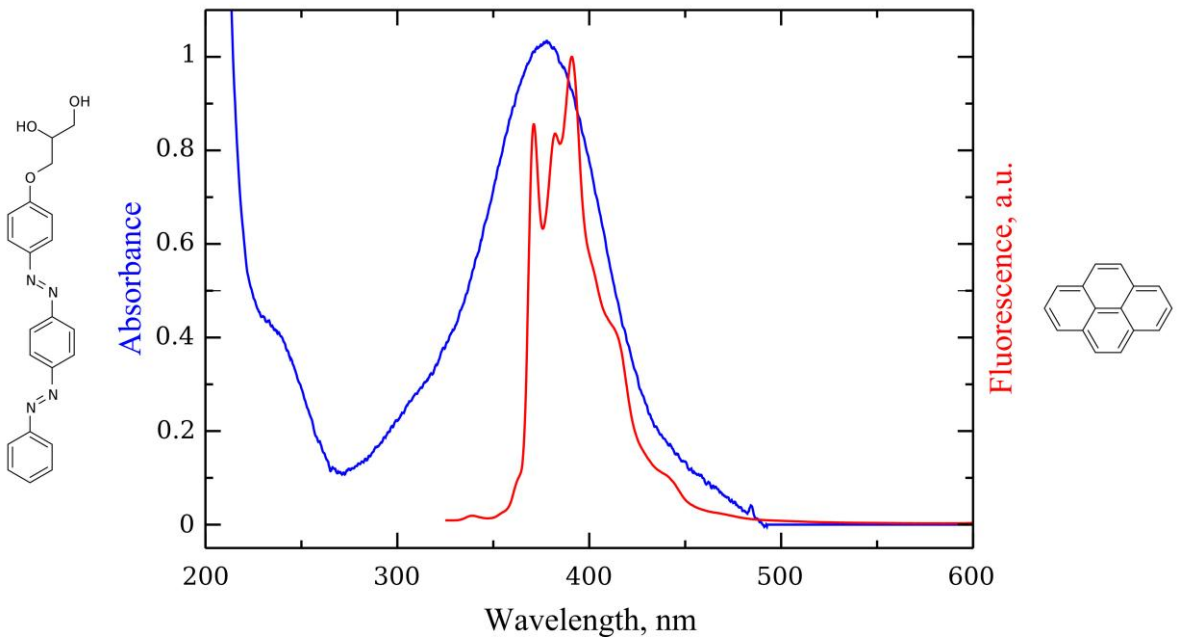


Figure 1. Spectral overlap. Blue: absorbance spectra of the title compound in ethanol. Red: emission spectra of the pyrene fluorophore in ethanol.

3. Materials and Methods

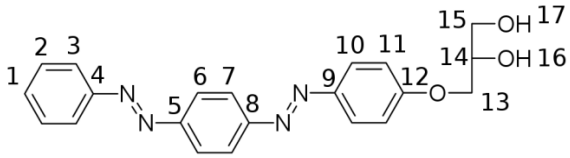
1,2-*O*-isopropylideneglycerol and triphenylphosphine were obtained from Sigma-Aldrich (St.Louis, MO, USA). Dianix yellow 5R (4-[(*E*)-{4-[(*E*)-Phenyldiazenyl]phenyl}diazenyl]phenol) was obtained from Reachem (Moscow, Russia). Diisopropyl azodicarboxylate (DIAD) was obtained from Acros (Geel, Belgium). All solvents were obtained from Ekos-1 (Moscow, Russia). Silica gel 60 (0.063–0.2 mm) and aluminum oxide 90 (basic, 0.063–0.2 mm) were obtained from Merck (Darmstadt, Germany). NMR spectra were recorded on Bruker Avance 700 MHz NMR spectrometer (Bruker,

Karlsruhe, Germany). Mass spectra was obtained on Bruker Maxis Impact ESI TOF mass-spectrometer (Bruker, Karlsruhe, Germany). UV-spectra was recorded on a LOMO SF-2000 spectrophotometer (LOMO, St. Petersburg, Russia). Fluorescence measurements were performed on Hitachi F-4000 spectrometer (Hitachi, Tokyo, Japan). Pyrene was excited at λex 310 nm, slits 5 nm each.

3-{4-[(E)-{4-[(E)-Phenyldiazenyl]phenyl}diazenyl]phenoxy} Propane-1,2-diol

Three hundred and two milligrams (1mmol) of 4-[(*E*)-{4-[(*E*)-phenyldiazenyl]phenyl}diazenyl] phenol, 262 mg (1 mmol) of triphenylphosphine, and 132 mg (1 mmol) of solketal were dissolved in 20 mL of THF and cooled on an ice bath. Then, 206 µL (1 mmol) of diisopropyl azodicarboxylate reagent were added. The mixture was left on the ice bath for 1 h, allowed to warm to room temperature, and left at that temperature for an additional 3 h. The reaction mixture was poured on a pad of silica gel (5 g) and washed out with chloroform. Concentration of the eluate on a rotary evaporating unit yielded 2 mL of a solution. The solution was poured on a pad of aluminum oxide (5 g) and washed out with chloroform. Evaporation of the eluate resulted in 260 mg (62%) of orange crystals. These were dissolved in 20 mL of THF and mixed with 4 mL of water and 2 mL of concentrated HCl. After 20 min, the reaction mixture was poured on 100 mL of water and extracted with 100 mL ethyl acetate (thorough mixing was needed). The organic solution was washed twice with water, dried over Na_2SO_4, and evaporated to yield 220 mg (93%) of the title compound as orange crystals. Mp 221–223 °C. ^{1}H-NMR (700 MHz, DMSO-d_6) δ 8.09 (2H, d, *J* = 8.2 Hz), 8.06 (2H, d, *J* = 8.4 Hz), 7.98–7.95 (4H, m), 7.66–7.59 (3H, m), 7.18 (2H, d, *J* = 8.5 Hz), 5.03 (1H, bs), 4.72 (1H, bs), 4.16 (1H, dd, *J* = 9.6, 4.2 Hz), 4.02 (1H, dd, *J* = 9.8, 6.1 Hz), 3.88–3.84 (1H, m), 3.49 (2H, d, J = 5.4 Hz). ^{13}C-NMR (176 MHz, DMSO-d_6) δ 162.61, 153.81, 153.12, 152.54, 146.78, 132.41, 130.04, 125.44, 124.27, 123.92, 123.22, 115.72, 70.65, 70.33, 63.06. ESI MS [M + H] found 377.1641, calculated 377.1608. ^{1}H and ^{13}C assignments are listed in Table 1. 1H, 13C, HMBC, HSQC and MS spectra are available as supplementary material.

Table 1. Chemical shift assignments.



Heavy Atom	^{1}H	^{13}C
1	7.62	132.4
2	7.64	130.0
3	7.95	123.2
4		152.5
5		153.8
6	8.06	123.9
7	8.09	124.2
8		153.1
9		146.7
10	7.96	125.4
11	7.18	115.7
12		162.6
13	4.02, 4.16	70.7
14	3.86	70.3
15	3.49	63.0
16, 17	5.03, 4.72	

Supplementary Materials: The following are available online: ^{1}H-, ^{13}C-NMR, HMBC, HSQC and MS spectra.

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Author Contributions: Both V.C. and I.B. have planned and performed experiments, analyzed data and written the manuscript. I.B. have synthesized title compound.

Conflicts of Interest: The authors declare no conflict of interest.

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